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14. ABSTRACT

Mild-to-moderate TBI is the signature injury of US military personnel. Thus, there is an urgent need to find biomarkers for mild-to-moderate TBI. In this proposal, our plan was to develop a "vasculome" database for TBI brain. By mapping the ***vasculome (i.e. entire gene expression profile in blood vessels)*** using mouse models of TBI, we will generate a comprehensive database that can be mined by the research community for potentially novel biomarkers of TBI. In this annual report, we describe our progress for the first year of our research, i.e. completion of all in vivo TBI mouse models, measurement of neurological deficits in all TBI mice, and the isolation of TBI microvessels and mRNA extraction.

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FINAL REPORT: MAPPING THE TBI VASCULOME

INTRODUCTION

Mild-to-moderate TBI is the signature injury of US military personnel. Thus, there is an urgent need to find biomarkers for mild-to-moderate TBI. In this proposal, our plan was to develop a "vasculome" database for TBI brain. By mapping the ***vasculome (i.e. entire gene expression profile in blood vessels)*** using mouse models of TBI, we will generate a comprehensive database that can be mined by the research community for potentially novel biomarkers of TBI. Our new approach is based on the fact that, in terms of surface area, the blood vessel endothelium is overwhelmingly the largest organ that secretes proteins into blood. Because the endothelium can survey the entire brain, they act as cellular integrators and sensors of brain dysfunction, releasing measurable biomarkers into the circulation. Thus, we propose that mapping the vasculome of stressed blood vessels in brain will generate a database for mining entirely novel TBI biomarkers in military personnel. We will build our vasculome database using mouse models of TBI - controlled cortical impact or closed head concussive injury. We cut away the core, i.e. directly damaged brain tissue. Then we extract blood vessels from non-directly-damaged ipsilateral hemisphere and contralateral hemisphere. Controls are collected from normal sham-operated brains. Samples are obtained at various times after TBI, ranging from initial injury (minutes to hrs) to delayed recovery (weeks). Total RNA is prepared and analyzed on the Affymetrix Mouse Whole Genome array. Perturbations are defined in vascular gene expression using GO and KEGG pathway analyses. Then the vasculome is mapped onto validated protein-protein interaction networks. A comprehensive database is generated and annotated, and all data will be open and freely accessible for the research community. In this final report, we describe our overall progress, i.e. completion of all in vivo TBI mouse models, measurement of neurological deficits in all TBI mice, and the isolation of TBI microvessels and mRNA extraction, building of the TBI vasculome.

BODY

Original Objectives and Deliverables:

Our project had three objectives. First, we would execute the two mouse models of TBI – open-cranium impact and closed-cranium impact, and perform a full assessment of neurologic deficits on standard behavioral tests post-TBI. Second, we would extract and characterize microvessel mRNA from ipsilateral and contralateral TBI brains, and confirm mRNA quality and microvessel purity. Third, we would then run all extracted microvessel mRNA post-TBI on Affymetrix gene arrays, and analyze the entire database with GO, KEGG and PPI networks to begin the definition of a TBI vasculome.

Final Findings:

We successfully met all expected milestones. In this final report, we report our findings as:

(a) two independent models of TBI were successfully performed with two different temporal profiles of neurologic deficits; (b) mRNA quality was established and high for all experiments; (c) brain microvessels were successfully isolated without contamination from blood elements or brain parenchyma; (d) the TBI vasculome was indeed perturbed compared to normal vasculome even in histologically normal-looking brain; (e) the TBI vasculome is reproducible when compared between independent experiments; (f) evolution of the TBI vasculome over time was different in controlled cortical impact versus closed-head impact models; (g) differentially expressed probes showed overlap and non-overlap between controlled cortical impact versus closed-head impact models; (h) pathway analysis also revealed both overlap and non-overlap between controlled cortical impact versus closed-head impact models.

a. Two independent models of TBI yielded two different temporal profiles of neurologic deficits

Over the course of the first few hrs to 21 days post-TBI, mice were evaluated with a battery of standardized tests, including the neurological severity score (NSS), the corner test, and foot-faults assessed with a wire-grip test. After open-cranium (standard controlled cortical impact) TBI, all mice developed severe neurological deficits that tended to be worst at 1-3 days post-injury, showed variable degrees of spontaneous recovery over time, but demonstrated remnant deficits even at the end of the observation period at 21 days **(Figure 1, see below)**. In contrast, mice subjected to closed-cranium TBI did not show severe deficits. Slight and variable deficits in NSS and foot-fault-wire-grip tests were recorded at 3 hrs post-injury, and all these rapidly renormalized so that by 21 days, mice appeared normal **(Figure 2, see next page)**.

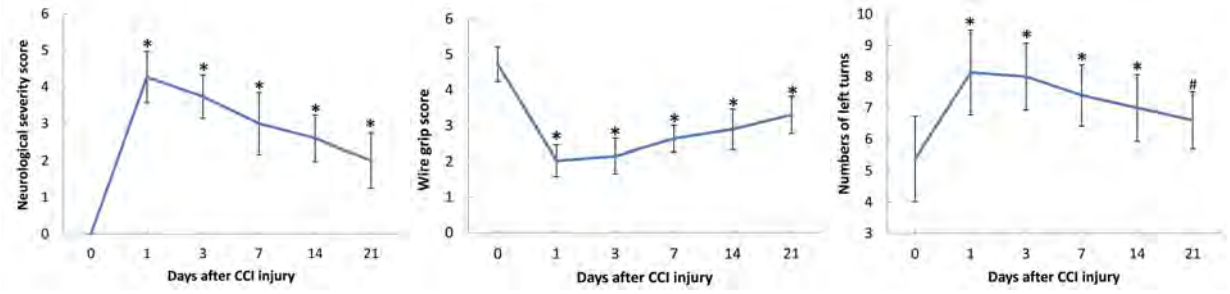

Figure 1

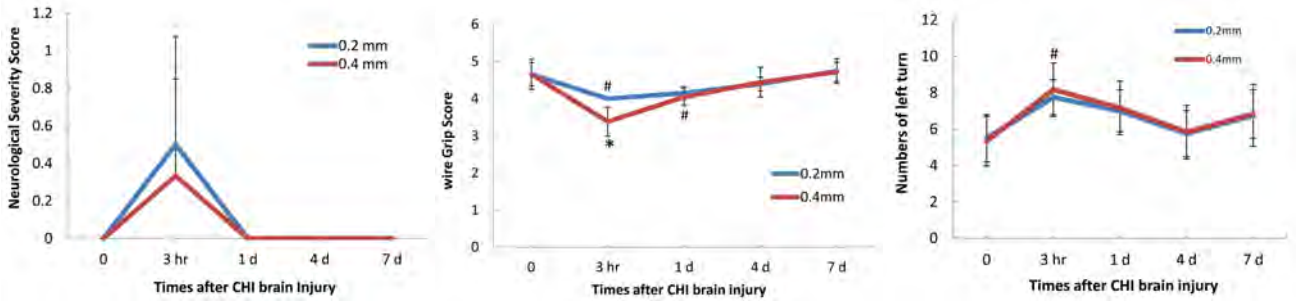

Figure 2

b. mRNA quality was established and high for all experiments

At each time point post-TBI, mice were killed and brains removed and microvessels were successfully purified from ipsilateral and contralateral hemispheres post-TBI in all mice. Based on these purified microvessels, mRNA was extracted using standard techniques.

In order to map the TBI vasculome, it is essential that two key quality controls steps are fulfilled. First, it is critical to demonstrate that mRNA quality is high enough for sufficient signal-to-noise to be obtained when samples are run on the full gene arrays. To accomplish this first step, mRNA quality in all samples was rigorously assessed with the Bioanalyzer method. We are pleased to report that mRNA quality passed this first quality-control step. Examples of Bioanalyzer runs are shown below (**Figure 3)**.

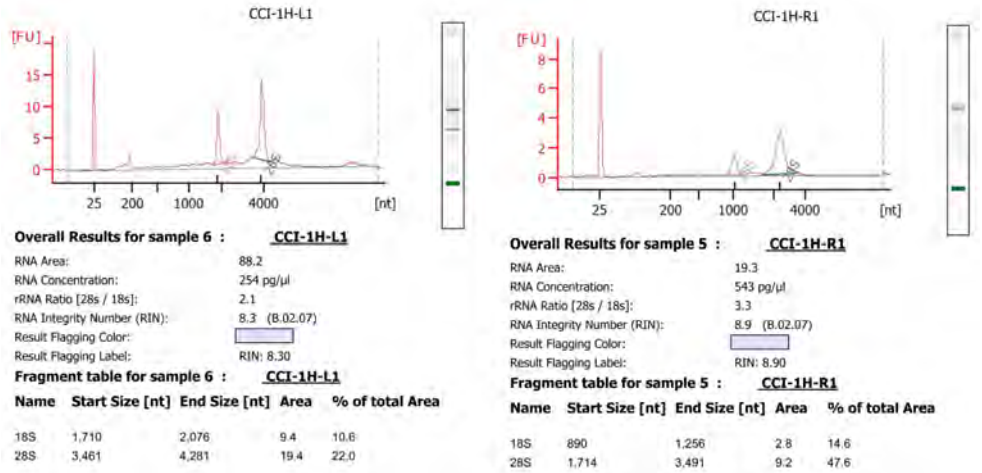

Figure 3

c. Brain microvessels were isolated without contamination from blood or brain parenchyma

To map the TBI vasculome, we also need to ensure that our microvessel samples are not contaminated by brain parenchyma (neuronal or glial components) or blood elements. In order to do this, we ran independent RT-PCR assays on all samples and compared the mRNA expression levels of representative endothelial genes versus neuronal and glial genes as well as blood cell genes. Endothelial genes were selected as cdh5, enos, pecam1. Neuronal genes were selected as neurogranin and map2. Astrocyte genes were selected as aqp4 and gfap. Blood cells markers were selected as Emr1, Mac1 and CD36. Our

RT-PCR data confirmed that our vasculome samples were indeed not contaminated by blood or brain **(Figures 4 and 5)**.

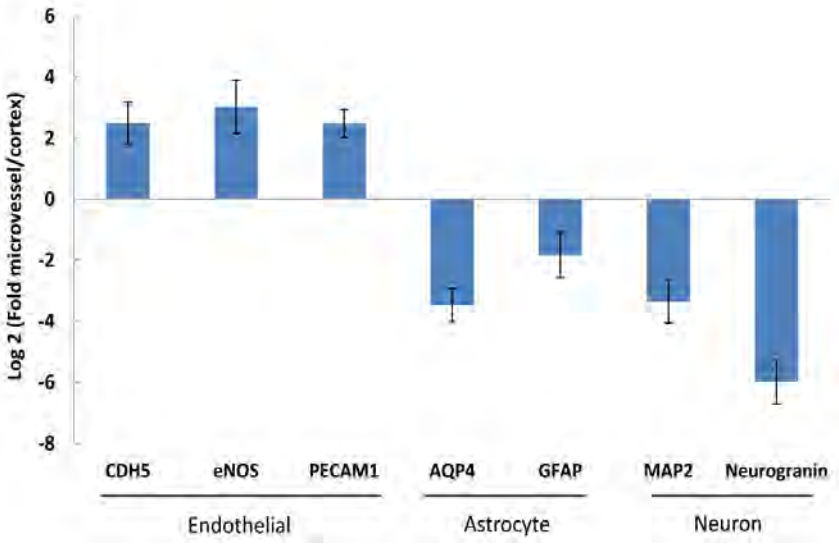

Figure 4

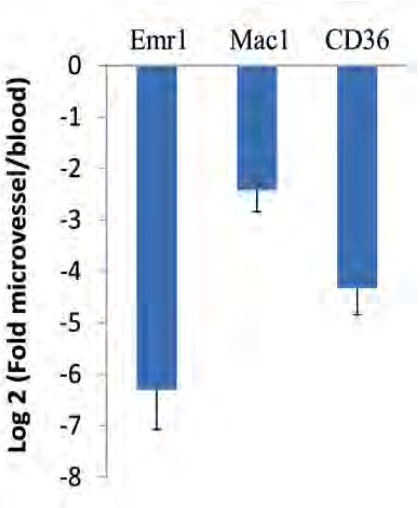

Figure 5

d. The TBI vasculome was perturbed even in histologically normal-looking brain

After TBI, a significant number of genes were altered in both TBI models. Table 1 below lists a summary of the numbers of genes that were upregulated or downregulated over time. Note: these responses occurred even in histoligically-normal looking brain tissue, suggesting that the TBI vasculome provides a source for pathogenic mechanisms as well as potential source for circulating biomarkers.

	CCI		CHI	
No.	upregulated	downregulated	upregulated	downregulated
1 hr	159	79	29	30
3 hr	221	114	12	46
6 hr	116	318	15	18
1 day	167	280	17	39
3 day	102	106	20	20
7 days	38	25	11	13
21 days	11	88	12	91

Table 1

e. The TBI vasculome is reproducible when compared between independent experiments

A central worry in all “large-data” studies is reproducibility. IN genomics and proteomics, it is not uncommon to find similar studies with different outcomes. So, an important part of our study was to assess reproducibility. We compared the TBI vasculome in two separate experiments using the open-cranium (controlled cortical impact) model. Note that the experiments were performed by different lab members, and analysis was performed in two separate batches of micro-arrays. Our studies found good concordance. Please see list of genes that were common between the two independent experiments (Table 2 next page). These data suggest that the TBI vasculome may be reproducible.

		First experiment, CCI		Second experiment, CCI		
symbol	entrezid	Probe ID	Ipsi/Norm	Probe ID	Ipsi/Norm	description
Serpina3n	20716	1419100_at	7.20	17278328	4.76	serine (or cysteine) peptidase inhibitor, clade A, member 3N
Adamts1	11504	1450716_at	5.71	17331705	3.90	a disintegrin-like and metallopeptidase (reprolysin type) with thrombospondin type 1 motif, 1
Timp1	21857	1460227_at	15.03	17533713	3.53	tissue inhibitor of metalloproteinase 1
Emp1	13730	1416529_at	5.66	17548559	3.40	epithelial membrane protein 1
Apln	30878	1451038_at	14.93	17541378	2.95	apelin
Tuba1c	22146	1448232_x_at	3.66	17314693	2.63	tubulin, alpha 1C
Chga	12652	1418149_at	2.61	17278090	2.44	chromogranin A
Sema4a	20351	1448110_at	2.63	17406760	2.33	sema domain, Ig domain, transmembrane domain and short cytoplasmic domain, (semaphorin) 4A
Angpt2	11601	1448831_at	6.15	17507799	2.22	angiopoietin 2
Cacna1g	12291	1423365_at	2.16	17267885	2.21	calcium channel, voltage-dependent, T type, alpha 1G subunit
C4b	12268	1418021_at	2.01	17343918	2.21	complement component 4B (Childo blood group)
Gfap	14580	1440142_s_at	4.17	17270354	2.20	glial fibrillary acidic protein
Tgfbi	21810	1456250_x_at	4.63	17287827	2.19	transforming growth factor, beta induced
Dot1l	208266	1457268_at	2.32	17235408	2.18	DOT1-like, histone H3 methyltransferase (S. cerevisiae)
Sulf1	240725	1436319_at	2.45	17211198	2.14	sulfatase 1
Ccl2	20296	1420380_at	2.80	17254041	2.01	chemokine (C-C motif) ligand 2
Ogn	18295	1419663_at	0.16	17287175	0.49	osteoglycin
Hmgcs2	15360	1431833_a_at	0.28	17400862	0.46	3-hydroxy-3-methylglutaryl-Coenzyme A synthase 2
Ecm2	407800	1440096_at	0.29	17287148	0.45	extracellular matrix protein 2, female organ and adipocyte specific
Slc40a1	53945	1448566_at	0.38	17222777	0.44	solute carrier family 40 (iron-regulated transporter), member 1
Prom1	19126	1419700_a_at	0.34	17447835	0.43	prominin 1
Rnf144b	218215	1443252_at	0.38	17287066	0.43	ring finger protein 144B
Car4	12351	1448949_at	0.36	17254508	0.43	carbonic anhydrase 4
Cxcl12	20315	1448823_at	0.34	17462149	0.42	chemokine (C-X-C motif) ligand 12
Slc22a8	19879	1416966_at	0.25	17357092	0.40	solute carrier family 22 (organic anion transporter), member 8
Itih5	209378	1441946_at	0.34	17366670	0.34	inter-alpha (globulin) inhibitor H5
Sema3c	20348	1429348_at	0.30	17434973	0.33	sema domain, immunoglobulin domain, short basic domain, secreted, (semaphorin) 3C
Ndrg1	17988	1423413_at	0.31	17312032	0.33	N-myc downstream regulated gene 1
Ptgds	19215	1423860_at	0.35	17382592	0.29	prostaglandin D2 synthase (brain)
Pltp	18830	1417963_at	0.31	17394297	0.27	phospholipid transfer protein
Slco1a4	28250	1420405_at	0.38	17472364	0.25	solute carrier organic anion transporter family, member 1a4
Itm2a	16431	1451047_at	0.29	17544078	0.24	integral membrane protein 2A

Table 2

f. Evolution of the vasculome over time was different in the two TBI models

A cluster analysis suggested that evolution of the vasculome over time was significantly different in the two independent models. In the open-cranium model, the TBI vasculome was most perturbed early on, and then showed signs of recovering over time. But in the closed-head model, the TBI vasculome appeared to progressively deteriorate over time. Please compare the cluster analysis graphs below **(Figure 6)**.

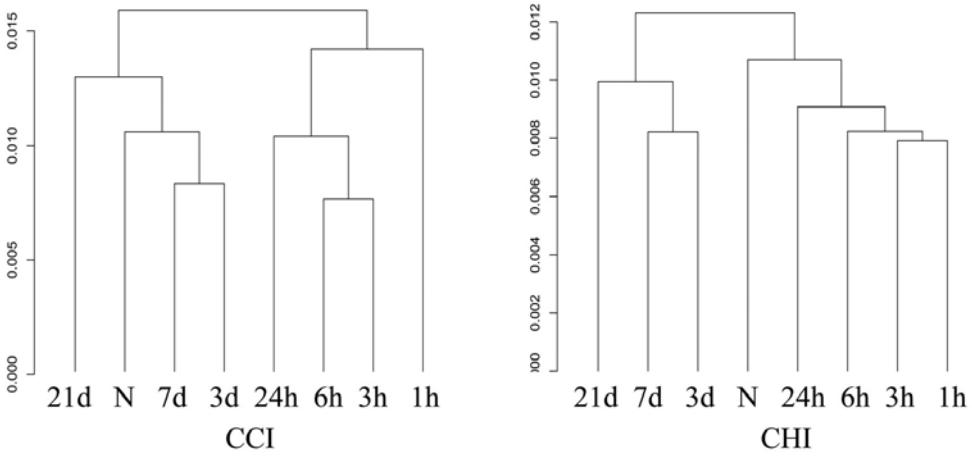

Figure 6

g. Differentially expressed probes show overlap and non-overlap between the two models

	CCI, 24 hr		CHI, 24 hr			
symbol	p	Ipsi/Norm	p	Ipsi/Norm	entrezid	description
Casq2	0.031	0.54	0.003	0.62	12373	calsequestrin 2
Cldn5	0.030	0.58	0.030	0.56	12741	claudin 5
Gm15567	0.029	1.78	0.020	1.66	100503685	predicted gene 15567
Higd1b	0.028	0.55	0.034	0.59	75689	HIG1 domain family, member 1B
Inha	0.031	1.51	0.026	1.52	16322	inhibin alpha
Irgm2	0.002	0.49	0.001	0.54	54396	immunity-related GTPase family M member 2
Ly6c1	0.022	0.58	0.023	0.58	17067	lymphocyte antigen 6 complex, locus C1
Ndrg1	0.006	0.33	0.038	0.55	17988	N-myc downstream regulated gene 1
Nek7	0.032	0.63	0.028	0.61	59125	NIMA (never in mitosis gene a)-related expressed kinase 7
Pltp	0.001	0.27	0.040	0.62	18830	phospholipid transfer protein
Ptprb	0.003	0.56	0.009	0.65	19263	protein tyrosine phosphatase, receptor type, B
Rgs5	0.010	0.58	0.014	0.63	19737	regulator of G-protein signaling 5
Sema3c	0.002	0.33	0.044	0.55	20348	sema domain, Ig domain, short basic domain, secreted, (semaphorin) 3C
Vtn	0.011	0.51	0.050	0.63	22370	vitronectin

Table 3, common differentially expressed genes in both CCI and CHI models, 24 hr post injury

	CCI, 24 hr		CHI, 24 hr			
symbol	p	Ipsi/Norm	p	Ipsi/Norm	entrezid	description
Abcg2	0.000	0.35	0.068	0.71	26357	ATP-binding cassette, sub-family G (WHITE), member 2
AI593442	0.002	0.39	0.245	0.85	330941	expressed sequence AI593442
Apln	0.038	2.95	0.012	1.35	30878	apelin
Bsg	0.003	0.37	0.043	0.70	12215	basigin
Cadm2	0.001	0.36	0.066	0.69	239857	cell adhesion molecule 2
Clec14a	0.047	0.37	0.117	0.56	66864	C-type lectin domain family 14, member a
Cxcl12	0.020	0.42	0.092	0.65	20315	chemokine (C-X-C motif) ligand 12
Emp1	0.023	3.40	0.318	0.72	13730	epithelial membrane protein 1
Gatm	0.023	0.37	0.120	0.61	67092	glycine amidinotransferase (L-arginine:glycine amidinotransferase)
Itih5	0.012	0.34	0.034	0.78	209378	inter-alpha (globulin) inhibitor H5
Itm2a	0.003	0.24	0.141	0.72	16431	integral membrane protein 2A
Mir138-1	0.020	2.63	0.133	1.44	387156	microRNA 138-1
Ptgds	0.023	0.29	0.464	0.63	19215	prostaglandin D2 synthase (brain)
Pxdn	0.026	2.77	0.533	0.92	69675	peroxidasin homolog (Drosophila)
Serpina3n	0.023	4.76	0.847	0.95	20716	serine (or cysteine) peptidase inhibitor, clade A, member 3N
Slc22a8	0.015	0.40	0.163	0.76	19879	solute carrier family 22 (organic anion transporter), member 8
Slco1a4	0.002	0.25	0.060	0.65	28250	solute carrier organic anion transporter family, member 1a4
Snord35b	0.005	0.39	0.117	0.78	27212	small nucleolar RNA, C/D box 35B
Timp1	0.013	3.53	0.947	0.99	21857	tissue inhibitor of metalloproteinase 1
Tuba1c	0.024	2.63	0.524	0.77	22146	tubulin, alpha 1C

Table 4, differentially expressed genes in CCI but not CHI model, 24 hr post injury

	CCI, 24 hr		CHI, 24 hr			
symbol	p	Ipsi/Norm	p	Ipsi/Norm	entrezid	description
BC033916	0.023	1.17	0.001	1.54	474160	cDNA sequence BC033916
Cdh5	0.281	0.78	0.038	0.56	12562	cadherin 5
Eng	0.175	0.72	0.023	0.45	13805	endoglin
Fn1	0.081	0.61	0.041	0.54	14268	fibronectin 1
Ftl1	0.101	0.69	0.049	0.58	14325	ferritin light chain 1
Ggt6	0.126	1.36	0.010	1.56	71522	gamma-glutamyltransferase 6
Gng3	0.639	0.91	0.010	1.53	14704	guanine nucleotide binding protein (G protein), gamma 3
Kdr	0.102	0.71	0.018	0.60	16542	kinase insert domain protein receptor
Lce3b	0.896	0.98	0.045	1.60	66344	late cornified envelope 3B
Mir124a-2	0.915	0.98	0.038	1.55	723950	microRNA 124a-2
Mir1949	0.606	0.90	0.025	0.56	100316700	microRNA 1949
Mir1955	0.677	0.89	0.040	0.60	100316756	microRNA 1955
Mir494	0.195	1.16	0.046	1.56	723878	microRNA 494
Myl9	0.197	0.46	0.049	0.55	98932	myosin, light polypeptide 9, regulatory
Rhox2b	0.061	1.20	0.007	1.59	100039913	reproductive homeobox 2B
Trav9d-3	0.571	0.92	0.012	1.57	100038850	T cell receptor alpha variable 9D-3
Txndc17	0.068	0.74	0.019	0.58	52700	thioredoxin domain containing 17
Vmn1r127	0.020	1.39	0.016	1.70	621561	vomeronasal 1 receptor 127
Vmn1r20	0.001	1.22	0.002	1.57	434017	vomeronasal 1 receptor 20

Table 5, differentially expressed genes in CHI but not CCI model, 24 hr post injury

	CCI, 21 day		CHI, 21 day			
symbol	p	Ipsi/Norm	p	Ipsi/Norm	entrezid	description
Arhgdia	0.037	0.56	0.049	0.66	192662	Rho GDP dissociation inhibitor (GDI) alpha
Atp1b3	0.047	0.62	0.029	0.54	11933	ATPase, Na+/K+ transporting, beta 3 polypeptide
Crip2	0.034	0.42	0.041	0.56	68337	cysteine rich protein 2
Ctnna1	0.026	0.62	0.020	0.66	12385	catenin (cadherin associated protein), alpha 1
Foxc1	0.041	0.64	0.026	0.65	17300	forkhead box C1
Kdr	0.038	0.47	0.047	0.63	16542	kinase insert domain protein receptor
Mfsd2a	0.018	0.54	0.045	0.66	76574	major facilitator superfamily domain containing 2A
Sema3c	0.036	0.45	0.047	0.62	20348	sema domain, Ig domain, short basic domain, secreted, (semaphorin) 3C
Sema7a	0.037	0.58	0.044	0.66	20361	sema domain, Ig domain, and GPI membrane anchor, (semaphorin) 7A
Syf2	0.027	0.61	0.017	0.64	68592	SYF2 homolog, RNA splicing factor (S. cerevisiae)
Timp3	0.047	0.59	0.031	0.57	21859	tissue inhibitor of metalloproteinase 3
Wfdc1	0.037	0.58	0.033	0.62	67866	WAP four-disulfide core domain 1
Zfp873	0.008	1.84	0.035	1.73	408062	zinc finger protein 873

Table 6, common differentially expressed genes in both CCI and CHI models, 21 day post injury

	CCI, 21 day		CHI, 21 day			
symbol	p	Ipsi/Norm	p	Ipsi/Norm	entrezid	description
Abcb1a	0.025	0.54	0.169	0.67	18671	ATP-binding cassette, sub-family B (MDR/TAP), member 1A
Abcc9	0.046	0.56	0.171	0.76	20928	ATP-binding cassette, sub-family C (CFTR/MRP), member 9
C1rb	0.022	1.61	0.109	1.37	667277	complement component 1, r subcomponent B
Capns1	0.019	0.57	0.104	0.78	12336	calpain, small subunit 1
Ece1	0.027	0.60	0.162	0.79	230857	endothelin converting enzyme 1
Emcn	0.037	0.45	0.214	0.72	59308	endomucin
Epas1	0.007	0.37	0.127	0.56	13819	endothelial PAS domain protein 1
Flt1	0.029	0.42	0.132	0.65	14254	FMS-like tyrosine kinase 1
Foxq1	0.008	0.46	0.083	0.73	15220	forkhead box Q1
H2afx	0.003	0.63	0.088	0.84	15270	H2A histone family, member X
Heg1	0.027	0.54	0.125	0.71	77446	HEG homolog 1 (zebrafish)
Jam2	0.041	0.57	0.142	0.69	67374	junction adhesion molecule 2
Nek7	0.036	0.63	0.295	0.76	59125	NIMA (never in mitosis gene a)-related expressed kinase 7
Pecam1	0.034	0.47	0.221	0.66	18613	platelet/endothelial cell adhesion molecule 1
Prom1	0.048	0.60	0.211	0.75	19126	prominin 1
Rgs5	0.035	0.51	0.216	0.80	19737	regulator of G-protein signaling 5
Stbd1	0.004	1.53	0.037	1.35	52331	starch binding domain 1
Wasf2	0.047	0.57	0.126	0.72	242687	WAS protein family, member 2
Zfp36	0.025	0.63	0.819	1.05	22695	zinc finger protein 36
Zfp85-rs1	0.043	1.51	0.285	1.34	22746	zinc finger protein 85, related sequence 1

Table 7, differentially expressed genes in CCI but not CHI model, 21 day post injury

	CCI, 21 day		CHI, 21 day			
symbol	p	Ipsi/Norm	p	Ipsi/Norm	entrezid	description
Gm5176	0.088	1.41	0.022	1.65	382421	high mobility group box 2 pseudogene
H2-T10	0.314	1.15	0.025	1.60	15024	histocompatibility 2, T region locus 10
Tnf	0.150	1.26	0.033	1.57	21926	tumor necrosis factor
Gm15363	0.067	1.32	0.002	1.57	669291	predicted gene 15363
Iqch	0.203	1.16	0.003	1.56	78250	IQ motif containing H
Egr3	0.419	1.10	0.012	1.52	13655	early growth response 3
Sprr2e	0.086	1.52	0.037	1.52	20759	small proline-rich protein 2E
Fbxw14	0.222	1.13	0.003	1.51	50757	F-box and WD-40 domain protein 14
Mir379	0.450	1.22	0.031	1.50	723858	microRNA 379
Ndrg1	0.098	0.56	0.040	0.55	17988	N-myc downstream regulated gene 1
Bag1	0.124	0.63	0.012	0.55	12017	BCL2-associated athanogene 1
Nid1	0.230	0.62	0.015	0.52	18073	nidogen 1
Rnu3b1	0.924	0.95	0.039	0.52	19858	U3B small nuclear RNA 1
Fam107a	0.125	0.59	0.021	0.49	268709	family with sequence similarity 107, member A
Wbp5	0.240	0.52	0.024	0.49	22381	WW domain binding protein 5
Snord58b	0.355	0.79	0.023	0.49	100217457	small nucleolar RNA, C/D box 58B
Imp3	0.197	0.49	0.029	0.43	102462	IMP3, U3 small nucleolar ribonucleoprotein, homolog (yeast)
Gatm	0.052	0.42	0.026	0.41	67092	glycine amidinotransferase (L-arginine:glycine amidinotransferase)
Mgp	0.147	0.53	0.032	0.34	17313	matrix Gla protein
Snord13	0.439	0.44	0.049	0.21	100217422	small nucleolar RNA, C/D box 13

Table 8, differentially expressed genes in CHI but not CCI model, 21 day post injury

h. Pathway analysis show overlap and non-overlap between the two models

		CCI-24H-L vs Norm		CHI-24H-L vs Norm	
NAME	SIZE	NES	p	NES	p
KEGG_GAP_JUNCTION	81	-3.68	0.000	-2.28	0.000
KEGG_ALZHEIMERS_DISEASE	144	-3.65	0.000	-2.03	0.002
KEGG_HUNTINGTONS_DISEASE	159	-3.30	0.000	-2.13	0.002
KEGG_VASCULAR_SMOOTH_MUSCLE_CONTRACTION	106	-2.58	0.002	-1.84	0.006
KEGG_REGULATION_OF_ACTIN_CYTOSKELETON	205	-1.98	0.008	-3.02	0.000
KEGG_AXON_GUIDANCE	128	-1.89	0.008	-2.36	0.000

		CCI-24H-L vs Norm		CHI-24H-L vs Norm	
NAME	SIZE	NES	p	NES	p
KEGG_OXIDATIVE_PHOSPHORYLATION	115	-4.03	0.000	-1.73	0.031
KEGG_PARKINSONS_DISEASE	110	-4.20	0.000	-1.52	0.068
KEGG_TIGHT_JUNCTION	129	-2.06	0.006	-1.48	0.071
KEGG_BASE_EXCISION_REPAIR	30	1.92	0.008	1.47	0.085
KEGG_CALCIUM_SIGNALING_PATHWAY	167	-2.03	0.004	-1.15	0.267

		CCI-24H-L vs Norm		CHI-24H-L vs Norm	
NAME	SIZE	NES	p	NES	p
KEGG_MTOR_SIGNALING_PATHWAY	51	0.63	0.924	-1.84	0.008
KEGG_TGF_BETA_SIGNALING_PATHWAY	83	-0.74	0.775	-2.03	0.004
KEGG_WNT_SIGNALING_PATHWAY	143	-1.20	0.234	-2.04	0.008
KEGG_NEUROTROPHIN_SIGNALING_PATHWAY	119	-1.50	0.070	-2.25	0.000
KEGG_LEUKOCYTE_TRANSENDOTHELIAL_MIGRATION	111	-0.85	0.655	-2.28	0.002
KEGG_MAPK_SIGNALING_PATHWAY	255	-1.17	0.240	-2.49	0.002
KEGG_UBIQUITIN_MEDIATED_PROTEOLYSIS	122	-0.91	0.560	-2.75	0.000
KEGG_CHEMOKINE_SIGNALING_PATHWAY	162	-1.54	0.054	-2.75	0.000
KEGG_ENDOCYTOSIS	171	1.39	0.097	-2.93	0.000
KEGG_FOCAL_ADHESION	192	-1.50	0.059	-3.47	0.000

Table 9, common changed pathways in CCI and CHI models, and CCI specific, CHI specific changed pathways, at 24 hr post injury

		CCI-21D-L vs Norm		CHI-21D-L vs Norm	
NAME	SIZE	NES	p	NES	p
KEGG_MAPK_SIGNALING_PATHWAY	255	-2.10	0.004	-1.76	0.008
KEGG_NEUROTROPHIN_SIGNALING_PATHWAY	119	-2.15	0.006	-2.56	0.000
KEGG_REGULATION_OF_ACTIN_CYTOSKELETON	205	-2.19	0.000	-2.02	0.008
KEGG_FOCAL_ADHESION	192	-2.29	0.000	-1.96	0.006
KEGG_ENDOCYTOSIS	171	-2.38	0.000	-2.95	0.000
KEGG_LYSOSOME	114	-2.42	0.000	-2.95	0.000
KEGG_GAP_JUNCTION	81	-2.71	0.000	-2.56	0.000
KEGG_CITRATE_CYCLE_TCA_CYCLE	30	-3.22	0.000	-2.50	0.000
KEGG_ALZHEIMERS_DISEASE	144	-4.20	0.000	-3.49	0.000
KEGG_PARKINSONS_DISEASE	110	-4.31	0.000	-3.77	0.000
KEGG_OXIDATIVE_PHOSPHORYLATION	115	-4.55	0.000	-4.26	0.000
KEGG_HUNTINGTONS_DISEASE	159	-5.20	0.000	-4.29	0.000

		CCI-21D-L vs Norm		CHI-21D-L vs Norm	
NAME	SIZE	NES	p	NES	p
KEGG_NEUROACTIVE_LIGAND_RECEPTOR_INTERACTION	259	2.65	0.000	1.73	0.032
KEGG_VASOPRESSIN_REGULATED_WATER_REABSORPTION	42	-1.98	0.006	-1.68	0.028
KEGG_ERBB_SIGNALING_PATHWAY	86	-1.98	0.008	-1.65	0.017
KEGG_APOPTOSIS	79	-2.12	0.002	-1.89	0.011
KEGG_UBIQUITIN_MEDIATED_PROTEOLYSIS	122	-2.38	0.000	-0.93	0.512
KEGG_AXON_GUIDANCE	128	-2.44	0.000	-1.70	0.027
KEGG_ADHERENS_JUNCTION	72	-2.75	0.000	-1.95	0.011

		CCI-21D-L vs Norm		CHI-21D-L vs Norm	
NAME	SIZE	NES	p	NES	p
KEGG_VEGF_SIGNALING_PATHWAY	73	-1.52	0.061	-2.04	0.004
KEGG_GLIOMA	59	-1.58	0.059	-2.12	0.006
KEGG_ACUTE_MYELOID_LEUKEMIA	57	-1.41	0.101	-2.14	0.000
KEGG_LEUKOCYTE_TRANSENDOTHELIAL_MIGRATION	111	-1.77	0.016	-2.48	0.000

Table 10, common changed pathways in CCI and CHI models, and CCI specific, CHI specific changed pathways, at 21 day post injury

KEY RESEARCH ACCOMPLISHMENTS

- Compared the temporal evolution of neurobehavioral outcomes in open-cranium versus closed-cranium cortical impact in male mice.

- Showed that high quality mRNA could be isolated in microvessels extracted from "morphologically-undamaged" ipsilateral brain tissue post-TBI, thus validating the feasibility of profiling the TBI vasculome.

- Showed that the TBI vasculome is reproducible in two independent experiments.

- Showed that open-cranium injury produces a "recovering" vasculome whereas closed-cranium injury may produce a "progressively worsening" vasculome over time.

- Showed that effects on the brain vsculome is dependent on the type of TBI involved – direct cortical injury or indirect closed-head injury.

- Demonstrated the overall feasibility of mapping pathways in the TBI vasculome.

REPORTABLE OUTCOMES

Manuscripts, abstracts, presentations:

- Guo S, Zhou Y, Xing C, Lok J, Som AT, Ning M, Ji X, Lo EH: The vasculome of the mouse brain. PLoS One. 2012; 7:e52665. PubMed PMID: 23285140; PubMed Central PMCID: PMC3527566.
  Note: this performance of experiments for this paper was completed before the start of the present USA MRAA funding. However, the concepts proposed herein provide the direct basis for our current TBI vasculome project.

- 8 Feb 2013: Thomas Willis Award Lecture, International Stroke Conference
  "Causation and collaboration for stroke research"
  Note: this lecture was more focused on stroke rather than TBI. However, the concept of the vasculome was presented and discussed.

- 26 Feb 2013: Invited lecture, Scientific Advisory Symposium, Biomedical Research and Integrative Neuroscience Center University of New Mexico
  "Mechanisms and challenges for translational neuroscience"
  Note: this presentation focused on the challenges of translation. The vasculome was presented as a model system whereby cellular mechanisms may lead to clinically relevant outcomes and endpoints.

- 4 April 2013: Neuroscience Grand Rounds, University of Louisville, Louisville, KY
  "Neurovascular mechanisms and challenges for translational research"
  Note: Lousville is considered one of the leading TBI research and clinical centers in the world. The presentation of the vasculome concept was well received.

- 27 April 2013: Plenary Session: Traumatic brain injury – journey to recovery. 20th Annual Meeting, American Society for Neural Therapy and Repair
  "Translational challenges for injury and repair: relevance to TBI"
  Note: The 2013 meeting of the ASNTR was focused on TBI. This presentation was given in a special session on military TBI, along with leaders in the field such as Dr. Ronald Hayes (University of Florida), Lt. Col. Christine Stahl (McDill Air Force Base), and Dr. Steven Scott (Chief of Rehab, VA Hospital, Tampa). The concept of the TBI vasculome was presented and well received.

- 1 Oct 2013: Special Session: Frontiers in translation neuroscience. 11th Annual Meeting, NeuroCritical Care Society
  "Neurovascular unit, MMPs and stem cells"
  Note: The NeuroCritical Care Society is the leading society of physicians responsible for taking care of TBI patients acutely. This presentation emphasized the importance of the TBI vasculome concept.

- 15 March 2014: Visiting professor lecture, Safar Center, University of Pittsburgh
  "Mechanisms for translational research in brain injury"
  Note: The Safar Center in Pittsburgh is a leading center for TBI science.

- 3 June 2014: Opening keynote lecture, 9th Hershey Conference on Developmental Brain Injury, "Cell-cell signaling in brain injury"

- 17 September 2014: Invited lecture, Montreal Neurological Institute,
  "Challenges and opportunities for translational research in brain injury"

- 21 September 2014: Invited lecture, NINDS-NHLBI Workshop, National Institutes of Health
  "Models and mechanisms for neurovascular research"
  Note: Introduced the TBI vasculome concept at an NIH meeting for the first time.

- 23 October 2014: Invited plenary lecture, 16th COSBID conference, Boston, MA
  "Cell-cell signaling in brain injury"
  Note: Introduced the TBI vasculome concept to this international brain injury consortium.

- Guo et al, the TBI vasculome (planned for submission to J Neurotrauma)

patents and licenses applied for and/or issued: none

degrees obtained that are supported by this award: none

development of cell lines, tissue, or serum repositories: none

informatics such as databases and animal models: not yet

funding applied for based on work supported by this award: none

employment or research opportunities applied for and/or received based on experience/training supported by this award: none

CONCLUSIONS

As hypothesized, we were able extract viable high-quality mRNA from the TBI vasculome in mice. And as hypothesized, closed-cranium TBI resulted in highly subtle and inconsistent neurological deficits compared to the more severe standard open-cranium TBI models. These two models may now give us an opportunity to model the highly challenging, wide-spectrum, subtle and highly variable responses that may be observed in our military personnel who suffer from mild-to-moderate TBI.

This project remains important because it examines, for the first time, the novel concept that microvessels in brain are perturbed during mild-to-moderate TBI, even in "normal-looking" ipsilateral brain tissue. Why is this significant? There may be three potential reasons.

First, if the microvessels are indeed perturbed, even in the absence of clear and consistent neurological deficits and outright morphological damage to the brain parenchyma, they may release signals into circulating blood. An immediate product of our project is the definition of this TBI vasculome – an open database that can be annotated and mined for potential biomarkers by the community.

Second, besides releasing signals into blood, the TBI vasculome may also be a mechanistically critical source of abnormal signals into the brain itself. Hence, even in the absence of clear and consistent neurological deficits and outright morphological damage to the brain parenchyma, TBI microvessels may release factors that perturb neuronal and glial function. This project has produced a dataset that can now be used to generate new mechanistic hypotheses for future TBI research. THis leads us to point number three below.

Finally, biomarkers are important, no question. But what this project has made us really excited about now, is the possibility of using the TBI vasculome to define novel mechanisms to explain why mild-to-moderate TBI can induce highly subtle neurological perturbations in the absence of clear and consistent neurological deficits and outright brain tissue damage. Although not part of this project, our lab has obtained pilot data using internal institutional funding (Massachusetts General Hospital) to show that perturbed cerebral endothelial cells may release microparticles that can activate microglia into potentially dangerous phenotypes. These activated microglia are very subtle and do not exhibit typical M1 characteristics. Beyond biomarkers per se, this aspect of the TBI vasculome may reveal new therapeutic targets. We now hope and plan to build and expand upon this project to develop a new set of experiments combining cell biology with in vivo TBI models to explore this new mechanism. We sincerely feel that there is a serious gap in knowledge now. Why do our military personnel suffer form these neurological symptoms after mild-to-moderate TBI in the absence of outright brain tissue damage? We believe that our vacsulome now points to an entirely new mechanism of pathophsyiology that should be further investigated. We hope to seek the help of this ahency to identify collaborations with blast injury model labs to test our ideas moving forward.

REFERENCES: none.

APPENDIX: none.